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Changes in Some Blood Parameters As a Function of Stage and Number of Lactation in Milking Buffalo

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THIS study describes the variation in the levels of some blood constituents of 30 lactating, non pregnant buffaloes differing in their lactation number (from the 1st to the 5th) and at various stages of lactation period (at 6, 12, 18 and 24 wks. post calving). The animals were kept permanently under shed and were fed the same conventional types of feed stuffs (which are commonly used under the confinement of animals) all year round. However, no grazing was allowed. During the experimental period, which extended for three consecutive seasons (fall, winter and early spring), all buffaloes were maintained under similar managerial and environmental conditions. The obtained results could be summarized in the following : Lactation number had a significant ($P < 0.01$) effect on a serum total protein, globulin, total cholesterol,lipids and calcium. Stage of lactation had a significant ($P < 0.01$) effect on serum calcium content with a tendency to decrease with advanced weeks of lactation. Both stage and number of lactation had no significant effect on the concentration of serum albumin, inorganic phosphorus, iron and zinc. Serum total protein and globulin tended to increase with lactation number. While, serum total cholesterol and lipids increased up to the 3rd lactation. Whereas, the levels of serum calcium dropped up to the 4th lactation. The means levels of all parameters measured herein are of most similar to those reported in the current veterinary literature. So, these findings are indicatives of good health, adequate plane of nutrition and mineral supply.

Key words : Buffaloes, lactation, stage, blood serum components.

Lactation impose a great nutritional and metabolic strain upon the animal (Blaxter, 1964). Further, age and stage of lactation are physical factors that influence some biochemical parameters of blood (Shaffer *et al.*, 1981). However, the chemical analysis of the blood enables nutritionists and veterinarians to use these information (called blood profile testing) to adjust the nutritional status

or to monitor health and disease both in individuals or in groups of animals. In cattle, immense amount of data pertaining to systematic changes in selected blood constituents in relation to age and stage of lactation has been well documented (Payne and Leech, 1964; Hartmann and Lascelles, 1965; Tumbleson *et al.*, 1973a,b; Roussel *et al.*, 1982 and Oltner & Berglund, 1983).

In Egypt, buffalo remain the back bone of animal wealth. However, reports deal with the effects of stage and number of lactation on the norms of blood parameters in buffalo are scanty. Hence, the present study has been arranged to fill the gap in our knowledge about this point.

Material and Methods

The experiment was carried out in the Experimental Farm of Milk Replacers Research Center for Buffalo, Faculty of Agriculture, Ain Shams University. Observations were obtained from 30 lactating non-pregnant buffaloes in their 1st to 5th lactation, respectively (6 per each). The animals were housed outdoor on a concrete slatted floors, kept tetherd with provision of feeding troughs under shade. The buffaloes were fed on dry feeds and concentrate which would be basic ingredients in feed lot rations. Hence, the ration was based on cubes of concentrate feed mixture formed of undecorticated cotton seed meal, 35%, coarse wheat bran, 33%, yellow maize, 22%, rice bran, 4%, molasses, 3%, calcium carbonate, 2% and table salt, 1%. In addition, berseem hay and rice straw were offered as cited in Table 1.

Daily rations were calculated according to the allowances recommended by El-Ashry (1981) and were adjusted every two weeks according to individual body weight (kg) and milk yield (kg/day). The buffaloes had free access to water three times daily.

Blood was drawn from the jugular vein into vacutainer tubes during the morning hr at 6, 12, 18 and 24 wks post calving. Serum was separated by centrifugation within 2 hr from the time of collection and stored at —20°C not more than one week. The following biochemical determinations were carried out : serum total protein by the method of Armstrong and Carr (1964), albumin, (Doumas *et al.*, 1971), total cholesterol (Watson, 1960), total lipids, (Frings *et al.*, 1972), calcium (Kamal, 1960) and inorganic phosphorus,

(Fiske and Sabbarow, 1925). Serum iron and zinc were determined with the help of (Perkins-Eimer) Atomic Absorption Spectrophotometer after suitable dilutions. Serum globulin concentrations were calculated as the differences between total protein and albumin concentrations. Representative samples from each ration ingredient were taken and analysed for Ca, P, Zn and Fe. The figures are presented in Table 1. Milk yield of the buffaloes was obtained from the records of the Experimental Farm. The data were subjected to statistical analysis of variance according to Snedecor and Cochran (1968).

TABLE 1. Nutritional values, Ca, P, Fe and Zn contents and average daily intake of each ration ingredient.

Item	Ration ingredient			
	Conc. feed mix.	Hay	Rice straw	
Starch value	55.00	33.00	20.00	
Digestible protein	12.00	8.00	0.00	
Av. daily intake, head, kg.	7.00	3.00	6.00	
(Ca), g/kg feed	11.28	9.94	3.18	
Av. daily intake, head, g.	78.96	29.82	19.08	
Total intake, g/day				127.86
(P), g/kg feed	3.07	1.80	1.08	
Av. daily intake, head, g.	21.49	5.40	6.48	
Total intake, g/day				33.37
(Fe), ppm*	189.00	150.00	31.00	
Av. daily intake, head, ppm	1323.00	450.00	186.00	
Total intake, ppm/day				1959.00
(Zn), ppm*	45.00	83.00	8.00	
Av. daily intake, head, ppm.	315.00	249.00	48.00	
Total intake, ppm/day				612.00

*ppm = Part per million.

Results and Discussion

Serum proteins

The significance of plasma proteins as the reserve supply of amino acids for use by body tissue in times of increased need, and protein depletion is well known.

In the present study, with increasing lactation number, serum total protein showed significant ($P < 0.01$) increase (Table 2). The same trend was observed by other investigators (Tumbleson *et al.*, 1973b; Shaffer *et al.*, 1981; Roussel *et al.*, 1982) as cows grow older. Blaxter, (1964) stated that lactation impose a great nutritional and metabolic strain on the animal. This may result in a marked acceleration of protein metabolism, largely due to the synthesis of proteins secreted into milk. The insignificant change of serum albumin and the significant ($P < 0.01$) increase of serum globulin from the 2nd lactation onwards (Table 2) are in accordance with the data of (Larson and Touchberry, 1959; Mylrea and Healy, 1968; Hewett, 1974; Rowlands *et al.*, 1977; Roussel *et al.*, 1982) in cattle.

It seems that the increase in serum total protein is merely a reflection of changes in gamma-globulin concentration with age. Gamma globulin in serum is also known to be increased in aged people (Haferkamp *et al.*, 1966). The insignificant effect of stage of lactation on serum total protein and globulin in buffaloes are in disagreement with the findings of (Mikulec, 1971; Rowlands *et al.*, 1975) in cattle. These authors stated that the most significant changes in serum total protein and protein fractions were confined to the periods near to either sides of calving On the other hand, Hewett (1974) noted that serum albumin was not affected by stage of lactation. This finding agrees with our results. The mean levels of serum total protein presented herein are comparable to those obtained by Hartmann and Lascelles (1965) in lactating dairy cows and are similar to the values (7.56, 3.40 and 4.16 g%) for serum total proteine albumin and globulin, respectively as reported by Hamza and Zein El-Abdin (1976) in dairy buffaloes.

TABLE 2. Effect of stage and number of lactation on serum total protein, albumin and globulin (g%) in lactating buffaloes ($\bar{X}\pm SE$).

Lactation No.	Weeks after parturition				Lactation over all
	6	12	18	24	$\bar{X}\pm SE$
			Total Protein		
1	6.98±0.07	7.13±0.16	7.08±0.30	6.99±0.18	7.04±0.11A,a
2	7.34±0.14	7.08±0.32	7.50±0.11	7.46±0.27	7.37±0.10C,b
3	7.20±0.16	7.40±0.27	7.36±0.24	7.63±0.19	7.42±0.10E,b
4	7.66±0.15	7.72±0.30	7.72±0.24	7.54±0.34	7.66±0.12B
5	8.10±0.24	8.00±0.28	7.81±0.10	7.82±0.19	7.93±0.10B,D,F
Stage overall $\bar{X}\pm SE$	7.44±0.10	7.44±0.14	7.45±0.11	7.43±0.11	
			Albumin		
1	3.71±0.30	3.82±0.20	3.25±0.33	3.53±0.20	3.53±0.14
2	3.83±0.23	3.67±0.24	3.42±0.26	3.18±0.18	3.51±0.12
3	3.38±0.17	3.56±0.25	3.54±0.35	3.95±0.34	3.61±0.14
4	3.16±0.29	3.31±0.11	3.71±0.23	3.53±0.19	3.29±0.09
5	3.41±0.09	3.49±0.27	3.28±0.06	3.50±0.13	3.42±0.07
Stage overall $\bar{X}\pm SE$	3.52±0.11	3.57±0.10	3.35±0.12	3.54±0.11	
			Glubulin		
1	3.26±0.28	3.31±0.36	3.83±0.42	3.53±0.09	3.53±0.15A
2	3.51±0.30	3.41±0.44	4.03±0.35	4.28±0.13	3.85±0.18a
3	3.88±0.22	3.84±0.24	3.82±0.35	3.68±0.37	3.80±0.08c,C
4	4.50±0.38	4.41±0.41	4.55±0.31	4.01±0.52	4.37±0.18B,b,d
5	4.69±0.17	3.86±0.17	4.53±0.15	4.31±0.17	4.51±0.08B,b,D
Stage overall $\bar{X}\pm SE$	3.91±0.16	3.86±0.17	4.10±0.16	3.92±0.14	

A,B,C,D,E Means with different superscripts are significantly different (1%).
a,b,c,d,e Means with different superscripts are significantly different (5%).

Serum total cholesterol and lipids

The significant ($P < 0.01$) increase of serum total cholesterol and lipids with lactation number (Table 3) are in contrary to the observations of Herricson *et al.* (1977) in cows, they found that although milk yield was higher in the 3rd lactation, the cholesterol level was lower than in the earlier lactations. In turn, Shaffer *et al.* (1981) and Roussel *et al.* (1982) reported that serum total cholesterol increased with increasing age due to stress of lactation and pregnancy. The mean values of serum total cholesterol recorded herein are comparable to the value (96.1 mg%) as measured by Lennon and Mixer (1957), in lactating dairy cows and are slightly higher than those reported by Salem (1980) in lactating buffaloes. In the present work the mean values of total lipids are ranged between the value (363.9 mg%) obtained by De Araujo and Birgel (1968) in shegoats and the values (311.8 and 339.0 mg%) as determined by O'Kelly (1968) in Brahman bulls and steers, respectively, The fluctuations in serum total lipids recorded herein throughout lactation season revealed insignificant difference.

Calcium (Ca)

The significant ($P < 0.01$) fall of serum Ca content as lactation progressed (Table 4) is in agreement with the findings of Rowlands *et al.* (1975), Oltner and Berglund (1983) in lactating cows. It may reflect a drain imposed on calcium pool due to its excretion in milk during the course of lactation. Since, a significant increase of milk calcium was recorded in milking buffaloes with the progress of lactation period (Abd El-Moneim and Aboulnaga, 1988). Also, the significant ($P < 0.01$) decline in serum Ca with lactation number confirms the data of Oltner and Berglund (1983). As a function of age, the decrease in serum Ca levels with increasing age is consistant with the findings of Payne and Leech (1964); Tumbleson *et al.* (1973a); Shaffer *et al.* (1981); Roussel *et al.* (1982) in cattle. In general, the mean levels of serum Ca obtained in this study are within the range (7.4-13.45 mg%) for blood serum Ca in buffaloes as reported by El-Naggar *et al.* (1979).

TABLE 3. Effect of stage and number of lactation on serum total cholesterol and total lipids (mg%) in lactating buffaloes ($\bar{X}\pm SE$).

Lactation No.	Weeks after parturition				Lactation overall
	6	12	18	24	$\bar{X}\pm SE$
			Total cholesterol		
1	78.6± 5.5	**71.4±12.3**	81.8± 9.8	**26.3±** 4.8	81.0± 3.9A,a
2	112.9±12.5	**94.6±** 8.6	103.0±10.7	**97.8±14.1**	102.5± 5.7b
3	103.5±13.4	**112.8±14.0**	117.8±16.5	**124.3±17.2**	114.4±7.2B
4	99.3±10.0	**114.6±11.2**	114.6±10.0	**98.2±10.7**	106.2± 5.0b
5	90.1±12.4	**101.8±** 7.0	93.6±14.6	**106.4±12.2**	97.9± 5.4
Stage overall $\bar{X}\pm SE$	99.3± 5.7	**100.3±** 5.9	101.1±14.6	**101.9±** 5.9	
			Total lipids		
1	243.7±28.0	**243.7±25.3**	234.0±11.9	248.8±12.1	242.2± 7.9B
2	314.2±20.8	**315.2±36.6**	329.3±16.7	**319.2±26.3**	320.2±11.5A,a
3	337.8±23.9	**371.2±31.5**	383.7±43.2	**389.7±25.9**	368.8±15.5A,b,c
4	349.3±39.1	**333.3±34.3**	361.0±17.4	**309.3±13.6**	338.2±13.4A
5	285.3±23.7	**313.7±21.9**	321.7±41.8	**333.3±31.5**	313.5±14.2A,d
Stage overall $\bar{X}\pm SE$	310.3±13.3	**321.6±16.6**	320.4±15.8	316.3±14.5	

TABLE 4. Effect of stage and number of lactation on serum calcium and inorganic phosphorus (mg%) in lactating buffaloes ($\bar{X}\pm SE$).

Lactation No.	Weeks after parturition				Lactation overall
	6	12	18	24	$\bar{X}\pm SE$
			Calcium		
1	14.3±1.7	13.4±2.1	11.5±1.2	10.3±0.9	11.9±0.7A,a
2	11.9±0.7	10.3±1.2	9.4±1.2	7.7±1.4	9.8±0.6b
3	10.8±1.2	10.1±1.2	8.6±1.1	9.0±1.2	9.7±0.6b
4	10.7±1.0	9.6±1.4	7.9±1.1	7.2±1.1	8.8±0.7B,c
5	13.4±0.0	11.8±0.6	10.4±0.4	9.4±0.4	11.2±0.5d
Stage overall $\bar{X}\pm SE$	12.0±0.5A	10.9±0.6	9.7±0.6B	8.8±0.5B	
			Inorganic Phosphorus		
1	5.4±0.1	4.9±0.1	5.3±0.7	5.5±0.2	5.3±0.2
2	5.6±1.0	6.3±0.5	6.1±0.7	6.8±0.7	6.2±0.4
3	7.4±1.4	5.4±0.7	6.5±0.9	7.4±0.5	6.7±0.5
4	6.3±1.6	5.7±0.4	6.1±1.1	6.2±0.4	6.1±0.4
5	5.7±0.4	5.5±0.4	5.5±0.2	5.1±0.3	5.4±0.2
Stage overall $\bar{X}\pm SE$	6.1±0.5	5.6±0.2	5.9±0.3	6.2±0.3	

Inorganic phosphorus (P_{in})

The insignificant effect of stage of lactation on serum P_{in} (Table 4) is consistent with the findings of (Rowlands *et al.*, 1975; Forar *et al.*, 1982) in dairy cows. Ali *et al.* (1985) found that plasma P_{in} content was lower in lactating buffaloes at 60-90 days post-partum than those at 91-120 days after parturition. Whereas, serum P_{in} was higher in lactating buffaloes from 60-120 days post calving than at 120-240 days (Dhoble and Gupta, 1986). These inconsistency might be due to dietary differences, since plasma P_{in} is dependent on phosphorus intake (Hewett, 1974).

In the current study serum P_{in} raised up to the 3rd lactation without any significance (Table 4). These results are in agreement with the findings of Akinsoyinu (1981) in lactating goats. In cattle, Forar *et al.* (1982); Olter and Berglund (1983, claimed that, first lactation cows have higher plasma P_{in} than multiparous cows. However, it is assumed that these variations may reflect nutritional and managerial differences. In general, the mean values of P_{in} presented herein are within the range (4.3 - 7.7 mg%) of serum P_{in} in lactating cows as reported by Rowlands *et al.* (1975).

Iron (Fe)

In view of the relatively large requirements for iron and its occurrence in major quantities in blood and muscles as well as in numerous enzymes, iron traditionally has been included among the essential trace minerals. (Hidiroglou, 1979). In the present work, the mean levels of serum Fe during the course of lactation are almost constant at all 4 sampling occasions (Table 5) and are close to the upper limit of the range (161.99 - 489.32 μg%) for serum Fe in buffalo calves as reported by Devaraj *et al.* (1985). From the *2nd* lactation onwards the mean levels of serum Fe cited herein are slightly increased without any significance (Table 5). Sharma and Prasda (1982) noticed an increase of blood iron concentration with the advancement of age.

TABLE 5. Effect of stage and number of lactation on serum iron and zinc (ppm) in lactating buffaloes (X+SE).

Lactation No.	Weeks after parturition				Lactation overall
	6	12	18	24	$\bar{X}\pm SE$
			Iron		
1	4.40±0.70	**4.70±0.49**	4.42±0.35	4.08±0.48	4.35±0.23
2	5.37±0.13	**4.89±0.23**	4.46±0.56	**5.06±0.30**	4.92±0.20
3	3.85±0.58	**4.56±0.62**	5.12±0.48	**5.53±0.19**	4.75±0.27
4	4.97±0.23	**4.42±0.88**	4.58±0.44	**5.07±0.31**	4.76±0.21
5	4.70±0.39	**4.72±0.36**	4.18±0.88	**4.98±0.37**	4.64±0.25
Stage overall $\bar{X}\pm SE$	4.65±0.23	**4.66±0.23**	4.55±0.23	4.89±0.19	
			Zinc		
1	2.90±0.37	**1.85±0.45**	2.02±0.86	2.65±0.49	2.37±0.23
2	3.03±0.13	**2.22±0.26**	2.49±0.27	2.27±0.47	2.52±0.16
3	2.42±0.40	**2.07±0.30**	2.48±0.34	2.19±0.19	2.27±0.15
4	3.15±0.32	**2.83±0.04**	2.60±0.06	2.02±0.29	2.65±0.16
5	3.17±0.81	3.30±0.82	2.52±0.41	3.13±0.93	3.03±0.34
Stage overall $\bar{X}\pm SE$	2.89±0.17	2.40±0.20	2.39±0.14	2.44±0.21	

Zinc (Zn)

Determination of zinc is of great importance for its role in some enzymatic function and some hormone activities appear to be correlated with zinc. The mean levels of Zn recorded in Table (5) are slightly higher than the value (2.04 ppm) reported by Ali *et al.* (1985) in buffalo. Meanwhile, the present findings are in accordance with the values of plasma Zn obtained by Mehta and Gangwar (1984) in lactating buffaloes. In relation to sampling occasions and lactation number, the results revealed no significant effect of both factors although the level of serum Zn was the highest in the 5*th* lactation. However, the value obtained by Sharma and Prasda (1982) for blood Zn in lactating buffaloes seems to be higher than that found herein (being 5.62 vs 3.03 ppm) respectively. Such differences may be due to differences in Zn content between whole blood and serum or due to difference in dietary Zn intake.

Milk yield

Within the period covered, average milk yield was the highest at 6 weeks after parturition and then declined afterwards in all buffaloes of different lactation numbers. Meanwhile, milk production was lower in first lactation buffaloes than those in subsequent lactations, and reached its peak at the 3*rd* lactation (Table 6).

TABLE 6. Average milk yield (kg/wk) of lactating buffaloes at various stages and numbers of lactation.

Lactation No.	No. of animals	Weeks after parturition			
		6	12	18	24
1	6	46.9	42.8	41.3	32.8
2	6	54.5	46.3	40.7	35.9
3	6	66.2	59.4	51.9	49.0
4	6	60.8	52.6	48.9	38.4
5	6	59.4	50.1	40.1	36.6

In the present study, serum P_{in}, total lipids and cholesterol varied in parallel to the changes in milk yield along the five lactation numbers. However, it is reasonable to suppose that with increasing milk yield, the increased concentrations of lipids are associated with the demands of the udder for fatty acids for glyceride synthesis. On the other hand, the continual increase of serum total protein and globulin with lactation number and regardless of milk yield may be are associated with age. In turn, serum Ca concentrations exhibited a marked reduction with increasing milk yield in multiparous buffaloes. Also, because the animals were kept under the same nutritional and managerial conditions with veterinary attention, the levels of serum Zn and Fe were nearly constant at various stages and numbers of lactation. Since, Cakala *et al.* (1972); Comar and Bronner (1962) claimed that any variation in blood minerals within reasonable limits can be influenced by dietary changes, environment and other management factors.

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التغيرات التى تحدث فى بعض مكونات دم الجاموس الحلاب تبعا للتغير فى مرحلة موسم الادرار

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أجريت هذه الدراسة لمعرفة التغيرات التى يمكن أن تحدث فى مستويات بعض مكونات الدم المأخوذ من ٣٠ جاموسه حلابه فى مواسم ادرار مختلفة (من الاول الى الخامس) وأثناء مراحل مختلفة من موسم الحليب (بعد ٦ ، ١٢ ، ١٨ ، ٢٤ اسبوع من تاريخ الولاده) . وقد استمرت التجربة لثلاثة مواسم مناخية متتالية (الخريف ، الشتاء ، بداية الربيع). فى اثناء هذه الفترة كانت الحيوانات تتعرض لنفس الظروف البيئيه والغذائية حيث كانت تعيش تحت مظلات وتتغذى على نفس الاعلاف الجافه طوال العام . ويمكن تلخيص النتائج المتحصل عليها فيما يلى :

١ ـ كان لموسم الادرار تأثيرا معنويا على مستويات كل من بروتين مصل الدم الكلى والجلوبيولين والكولستيرول الكلى والدهون الكليه وكذلك الكالسيوم .

٢ ـ أثرت مرحلة الحليب تأثيرا معنويا على مستوى كالسيوم الدم حيث كان تركيزه ينقص مع التقدم فى موسم الحليب .

٣ ـ لم يكن لكل من موسم أو مرحلة الحليب أى تأثير معنوى على محتوى مصل الدم لكل من الالبيومين والفوسفور غير العضوى والحديد والزنك .

٤ ـ كلما زاد رقم موسم الحليب ازدادت مستويات بروتين مصل الدم الكلى والجلوبيولين بينما ازدادت مستويات الكولستيرول والدهون الكليه حتى الموسم الثالث للادرار . ومن ناحية أخرى أظهرت مستويات الكالسيوم تناقصا مستمرا حتى موسم الحليب الرابع .

٥ ـ لوحظ ان التغير فى محتويات مصل الدم من الفوسفور غير العضوى والكولستيرول والدهون الكليه كان مواكبا للتغير الذى حدث فى معدلات انتاج اللبن من موسم حليب الى آخر .